Character Association Studies in Various *Brassica Napus* Genotypes under Drought Stress

ABSTRACT

Increased aridity and desertification are one of the most threatening setbacks to present day agriculture throughout the globe. Pakistan, being a sub-tropical country, is on the verge of an overwhelmingly increased rate of desertification. Therefore, it needs abrupt interventions to ensure food security. *Brassica napus*, being an integral part of indigenous edible oil production in Pakistan, needs some serious interventions for its adaptability under rain-fed agro-ecological conditions. Therefore, the present investigation was carried out to screen drought tolerant *B. napus* genotypes. For that purpose, ten *B. napus* accessions (Shiralle, DGL, Cyclone, Punjab Sarsoon, Cone-1, Cone-2, Rainbow, Dunkled, Zn-R-8, and Zn-M-6) were screened out for their relative tolerance to drought stress both in in-vitro and in-vivo conditions. An in-vitro experiment was carried out by following a completely randomized design (CRD) with three replications. Polyethylene glycol (PEG) solution with various concentrations (T_1 = 2.5 % and T_2 = 5 %) was applied to induce drought conditions and was compared with the control treatment (T_o = normal). Data were recorded for seedling length, root length, number of secondary roots, fresh seedling weight, and dry seedling weight. Results of ANOVA elucidated that all varieties showed significant responses in accordance with all studied characters except root length. Correlation analysis revealed that seedling length exhibits a high positive correlation with dry shoot weight followed by that root length under drought conditions; while path analysis revealed maximum positive direct effects of dry shoot weight under drought conditions (both in T_1 and T_2). An in-vivo experiment was also conducted by following a randomized complete block design (RCBD) under split plot arrangement. Data were recorded on the following parameters; days to 50% emergence, plant height, leaf area, silique length, number of silique plant, number of seed/siliques, seed yield/plant and 1000-seed weight. Results of the in-vivo experiment based on correlation analysis revealed that plant height exhibits the highest positive correlation with 1000 seed weight followed by the number of seeds per silique. Path analysis revealed that seeds per silique showed maximum direct contribution in the seed yield per plant of all of the evaluated varieties under both drought treatments (T_1 and T_2). Among all of the evaluated varieties, varieties named “Cone-2” and “Zn-M-6” showed the highest performance under both drought and control conditions. Therefore, these genotypes are recommended for cultivation in arid agro-ecological zones of Pakistan.

Keywords: Brassica napus; Drought; Correlation; Path coefficient analysis

1. INTRODUCTION

Brassica (*Brassica napus*) is an important oilseed crop grown worldwide on large scale. Brassica has been ranked 4^{th} among the oilseeds crops in Pakistan. In Pakistan the total cultivated area under brassica is about 272, 100 ha, and production are about 230, 000 tons each year [1]. The average yield from a particular cultivated area is about 812 kg/ha. The tropical and subtropical regions of Pakistan are major areas for brassica production. Drought is the major climatic factor that play a significant role in the reduction of brassica production [2].

Drought not only reduces the yield per hectare but also the oil production. Non-availability of a proper irrigation system in major areas of Sindh and KPK results in low production and low yield. Drought is well known to trigger the growth and development of brassica. Plants display a range of mechanisms to withstand drought stress [3,4].

The primary processes involve reduced evaporation due to greater diffusive barriers, higher water intake due to productive and extensive root structures and effective use of this water, and small fleshy leaves to minimize transpiration [5]. With the passage of time, many novel techniques have been introduced in order to increase production under drought. Under stress, the production of reactive oxygen species (ROS) takes place, where free oxygen radical reacts with the normal chemical reactions of plants and disturb the chemical processes [6].

Pakistan requires 1.95 million tons of edible oil per year. Only 29% of this amount is satisfied by local resources, with imports accounting for the remaining 71% [7]. As a consequence, edible oil imports consume a considerable portion of the national budget ($900 million per year) [4]. As a consequence, boosting the yield of oilseed crops is important to bridging the gap. Brassicas are a major oilseed crop in our nation, accounting for around a quarter of total edible oil output. Brassicas, as native species (*Brassica napus*), have a strong potential to combat the situation caused by the introduction of high-quality canola. Drought is a key element that restricts the amount of land that can be cultivated and the production of crops. Canola was evolved in locations with a lot of rain and performs badly in areas with little rain [1,2]. Drought has also been noticed in irrigated regions owing to a lack of water supply and canal blockage. Plants respond to water stress by diminishing cell turgor, shutting stomata, shrinking cell expansion, and reducing leaf surface area, among other physiological changes. All of these anomalies limit photosynthesis and respiration [8,9], resulting in decreased crop output. To improve the area and output of oilseed crops, it is critical to develop cultivars that can withstand water stress. The current research was designed to accomplish these objectives by identifying drought-tolerant canola cultivars and estimating correlations among several features. The findings will aid in the breeding of Brassicas for drought tolerance, as well as the early identification of genotypes with favorable features for use in breeding programs.

2. MATERIAL AND METHODS

Two experiments were conducted in the plant breeding and genetics lab and farm. The first experiment was conducted under factorial CRD design and the second under split plot RCBD design. The data of both experiments were recorded and analyzed. Analysis of variance (ANOVA), mean comparison test (Tuckey test) was used to determine the variability in the genotypes with the help of statistics 8.1 software, correlation to determine the relationship in traits and check the direct and indirect effect with path analysis by the help of R-software.

The experimental material is comprised of ten varieties of *Brassica napus* L. (Shiralle, DGL, Cyclone, Punjab Sarsoon, Cone-1, Cone-2, Rainbow, Dunkled, Zn-R-8, and Zn-M-6). At maturity, ten plants per replication of each variety in each treatment were randomly marked, and data on various yield-related components (Plant Height (cm), Number of seeds per siliqua, Seed Yield per Plant, Days taken to 50% maturity, 1000 Seed Weight) were recorded for the lab experiment (Plant Height (cm), Number of seeds per siliqua, Seed Yield per Plant, Days taken to 50% maturity, 1000 Seed Weight). The data from the experiment included seedling length, shoot length, root length, fresh shoot weight, and dry shoot weight.

The siliquae harvested from designated plants were manually threshed and the seed recovered was weighed in grams using an electric balance to calculate seed production per plant. During mid-day, observations were taken from the lower surface of the leaf near the petiole, avoiding the midrib. After 50 percent flowering appeared on the marked plants, the number of days to flowering was recorded, and the number of days to siliquae formation was recorded after 50 percent siliquae appeared on the marked plants. From the time of sowing until 50% of the plants were mature, the number of days to 50% maturity was recorded. The maturation of siliquae was determined by the change in color from green to brown. The plant was judged mature when 50% of the siliquae became brown. An analysis of variance was performed on the data collected [10]. Differences in the performance of single cross hybrids under normal settings were also discovered. The genotypic, phenotypic, and environmental covariances were used to determine the character associations represented by the correlation coefficient between distinct pairs of characters at the genotypic and phenotypic levels. The term "covariance" refers to the tendency of two variables or characters that are related to each other to change at the same time, i.e. to change at the same time. The variance analysis was followed by the covariance analysis in the same way. The expectation for the mean product of the covariance analysis was similar to the expectation for the mean square of the analysis of variances. The estimates of the genotypic and phenotypic covariance components between two traits were therefore derived in the same way as the corresponding variance components. We followed the guidelines for genotypic and phenotypic association [11].

3. RESULTS AND DISCUSSION

The varieties were significantly different for all the traits except for root length (Table 1). Among the recorded traits the variability for seedling length (cm), shoot length (cm), root length (cm), fresh shoot weight (g), dry shoot weight (g), plant height (cm), days to 50% flowering, number of seeds per silique, seed yield per plant and 1000-seed weight ranged from 10.408-14.058, 6.2417-9.8333, 5.4333-6.9717, 0.984-1.2212, 0.41-0.6367, 60.78-127.83, 51.284-65.741, 95.3-109.31, 13.859-18189, 1.7344-3.4889 respectively. Treatments were considerable for every character under study. G x T interaction was significant only for fresh shoot weight. Under normal conditions, varieties exhibited significant differences for all the plant traits studied except plant height (Table 2).

In the present study, the effects of drought were evaluated on 10 different genotypes of *Brassica napus*. Drought stress reduced the plant height of 8 genotypes at a significant level and two genotypes (Cone-2 and Zn-M-6) showed better plant height under drought stress as compared to control conditions. Days to 50% flowering were greatly influenced by the drought stress of every plant under drought stress. Under control conditions, all the plants showed 50% of flowering after 8 weeks of germination whereas, during drought stress 50% flowering was observed after 10 and 11 weeks of germination under treatments T_1 and T_2 respectively.

The number of seeds per silique was also badly affected by drought stress. Under control conditions, the number of seeds per silique was better as compared to drought stress treatments. However, two genotypes (Cone-2 and Zn-M-6) performed well under drought stress in all treatments in each parameter during an experiment. Seed yield per plant and seed quality are highly influenced by both biotic and abiotic stresses. In the present study, seed yield per plant was very low under drought stress for eight genotypes as compared to control. However, the two genotypes (Cone-2 and Zn-M-6) seed yield was also reduced but not very much as compared to the other 8 genotypes. 1000-seed weight was normal under control conditions but gradually declined under drought stresses in each genotype except (Cone-2 and Zn-M-6).

Drought stress is one of the main factors known to reduce oil quality and seed yield of *brassica napus* per hectare. In the present study, different parameters were studied and each parameter of each genotype was highly affected during drought stress except (Cone-2 and Zn-M-6). Although, drought influenced the growth and development of each genotype. Drought stress as a combination of osmotic and ionic stress adversely influences the physiological and biochemical processes of the plants. These two (Cone-2 and Zn-M-6) genotypes should be taken for further breeding programs for more improvement against stresses.

3.1 Correlation and path analysis during lab experiment

Dry shoot weight was highly significant and positively correlated with FSW(r=0.019), (r=0.262) in both treatments T_1 and T_2. Path coefficient analysis revealed the results that FSW had a positive direct impact on the DSW during T_2. SH, SL, and FSW had a direct and positive impact on the DSW during T_2.

3.2 Correlation and path analysis during field experiment

Seed yield for each plant was significantly and positively associated with SPS (r=0.98167) and 1000-SW (r=0.43293) during $T_{0.}$ 1000-SW (r=0.43819), (r=0.43819) was positively correlated with seed yield per plant in both treatments T_1 and T_2. Path coefficient analysis revealed that SPS, 1000-SW, and SYPP had a direct and positive impact on the seed yield of *Brassica napus* during all treatments.

Table 1. Mean squares and their significance from analysis of variance of different plant traits under first experiment.

Characters	Mean square	Treatment (T)	G x T	Error	Minimum Range	Maximum Range
Seedling length	8.66361**	9.996**	0.26339	127.844	10.408	14.058
Shoot length	10.9728**	22.302**	1.9171	189.563	6.2417	9.8333
Root length	1.26663	5.934**	1.12313	65.5109	5.4333	6.9717
Fresh shoot weight	0.0360**	0.1217**	0.02042*	0.00877	0.984	1.2212
Dry shoot weight	0.03216**	0.06667**	0.00913	0.00685	0.41	0.6367

Table 2. Mean squares and their significance from analysis of variance of different plant traits under field experiment.

Characters	Mean square	Treatment	G x T	E_1	E_2	Minimum Range	Maximum Range
Plant height	0.59	937.08**	5.77**	0.78	0.49	60.78	127.83
Days to 50% flowering	0.163**	740.442**	4.985**	0.519	0.345	51.284	65.741
No. Of seed per silique	1.257**	783.22**	1.126**	0.232	0.332	95.3	109.31
Seed yield per plant	21.016**	654.814**	1.358	1.18	1.149	13.859	18.189
1000-seed weight	2.56148**	28.2559**	0.124**	0.022	0.009	1.7344	3.4889

Table 3. Phenotypic (lower) and genotypic (upper) correlation coefficients under stress of T_1 in *Brassica napus* L. genotypes.

	SL	RL	FSW	DSW
SH	-0.4980 -0.4950	0.3712* 0.3701*	-0.08971 -0.08965	0.6549 0.6528
SL		0.279* 0.280*	-0.0565* -0.0565*	-0.008** -0.008**
RL			-0.0878 -0.0873	0.5979 0.5970

FSW				0.019** 0.019**

SH= Seedling height, **SL=** Shoot length, **RL=** Root length, **FSW=** Fresh shoot weight, **DSW=** Dry shoot weight

Table 4. Phenotypic (lower) and genotypic (upper) correlation coefficients under stress of T_2 in *Brassica napus* L. genotypes.

	SL	**RL**	**FSW**	**DSW**
SH	0.0953* 0.0951*	0.516* 0.513*	-0.4869 -0.4850	0.1033 0.1036
SL		0.033* 0.033*	-0.579 -0.576	-0.395 -0.394
RL			-0.101 -0.101	0.295 0.296
FSW				0.262* 0.262*

SH= Seedling height, **SL=** Shoot length, **RL=** Root length, **FSW=** Fresh shoot weight, **DSW=** Dry shoot weight.

Table 5. Direct (Bold) and indirect effects of drought stress treatment (T_1) on dry shoot weight in *Brassica napus* L. genotypes

	SH	**SL**	**RL**	**FSW**	**DSW**
SH	**-0.1373**	0.0343	-0.00611	0.0492	0.1167
SL	0.0684	**-0.0689**	-0.0044	0.0311	-0.0015
RL	-0.0509	-0.0194	**-0.0163**	0.0483	0.1065
FSW	0.0121	0.0037	0.00143	**-0.5512**	-0.0036
DSW	-0.0899	0.0004	-0.0096	0.00117	**0.1782**

SH= Seedling height, **SL=** Shoot length, **RL=** Root length, **FSW=** Fresh shoot weight, **DSW=** Dry shoot weight

Table 6. Direct (Bold) and indirect effects of drought stress treatment (T_2) on dry shoot weight in *Brassica napus* L. genotypes

	SH	**SL**	**RL**	**FSW**	**DSW**
SH	**0.080**	0.0368	-0.1830	-0.480	-0.008
SL	0.0076	**0.385**	-0.01254	-0.574	-0.038
RL	0.040	0.011	**-0.352**	-0.1024	-0.028
FSW	-0.038	-0.223	0.034	**0.988**	-0.017

DSW	0.0083	-0.1536	-0.1056	-0.262	**0.98**

SH= Seedling height, **SL=** Shoot length, **RL=** Root length, **FSW=** Fresh shoot weight, **DSW=** Dry shoot weight

Table 7. Phenotypic (lower) and genotypic (upper) correlation coefficient among different parameters in *Brassica napus* L. genotypes under normal condition (T_0)

	D50.F	SPS	SYPP	1000-SW
PH	0.011 -0.0132	-0.1369 -0.1967	-0.1452 0.03681	0.52295** 0.56413**
D50.F		0.98167** 0.96806**	-0.7391** -0.426**	0.48732** 0.42059*
SPS			-0.7198** -0.4789**	0.43293** 0.42059
SYPP				-0.391* -0.0963

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

Table 8. Phenotypic (lower) and genotypic (upper) correlation coefficient among different parameters in *Brassica napus* L. genotypes under drought stress treatment (T_1)

	D50.F	SPS	SYPP	1000-SW
PH	-0.1673 -0.1666	-0.1336 -0.133	-0.0425 -0.0423	0.62072** 0.60537
D50.F		0.89669 0.88681	0.10507 0.10391	0.34782* 0.32895
SPS			-0.2106 -0.2032	0.43819** 0.42709**
SYPP				0.0362 0.0321

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

Table 9. Phenotypic (lower) and genotypic (upper) correlation coefficient among different parameters in *Brassica napus* L. genotypes under drought stress treatment (T_2)

	D50.F	SPS	SYPP X	1000-SW
PH	-0.1673 -0.1666	-0.1336 -0.133	-0.0425 -0.0423	0.62072** 0.60537**
D50.F		0.89669 0.88681	0.10507 0.10391	0.34782* 0.32895
SPS			-0.2106 -0.2032	0.43819** 0.42709**
SYPP				0.0362 0.0321

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

Table 10. Direct (Bold) and indirect effects of different parameters on seed yield per plant of *Brassica napus* L. genotypes under normal condition (T_0)

	PH	D50.F	SPS	SYPP	1000-SW
PH	**-0.3479**	-0.0143	-0.0968	0.02676	0.03007
D50.F	-0.0038	**-1.3003**	0.6941	0.13619	0.02802
SPS	0.04762	-1.2764	**0.70706**	0.13263	0.02489
SYPP	0.05052	0.96099	-0.5089	**-0.1843**	-0.0225
1000-SW	-0.1819	-0.6336	0.30611	0.07205	**0.05749**

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

Table 10. Direct (Bold) and indirect effects of different parameters on seed yield per plant of *Brassica napus* L. genotypes under drought stress treatment (T_1)

	PH	D50.F	SPS	SYPP	1000-SW
PH	**-0.1255**	0.24839	-0.2196	-0.0206	-0.344
D50.F	0.021	**-1.4848**	1.47414	0.05098	-0.1928
SPS	0.01677	-1.3314	**1.64398**	-0.1022	-0.2428

SYPP	0.00533	-0.156	-0.3463	**0.48522**	-0.0201
1000-SW	-0.0779	-0.5164	0.72037	0.01757	**-0.5542**

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

Table 11. Direct (Bold) and indirect effects of different parameters on seed yield per plant of *Brassica napus* L. genotypes under drought stress treatment (T_2)

	PH	**D50.F**	**SPS**	**SYPP**	**1000-SW**
PH	**-0.3479**	-0.0143	-0.0968	0.02676	0.03007
D50.F	-0.0038	**-1.3003**	0.6941	0.13619	0.02802
SPS	0.04762	-1.2764	**0.70706**	0.13263	0.02489
SYPP	0.05052	0.96099	-0.5089	**-0.1843**	-0.0225
1000-SW	-0.1819	-0.6336	0.30611	0.07205	**0.05749**

PH= Plant height, **D50%F=** Days to 50% flowering, **SPS=** Seeds per silique, **SYPP=** Seed yield per plant, **1000-SW=** 1000-seed weight

4. CONCLUSION

Water stress resulted in significant decreases in nearly all of the traits examined. It was found that the siliques quantity in each plant dropped by the most dramatic margin. More siliques led to a higher seed output, whereas fewer siliques on each plant led to a lower seed yield [12,13]. Therefore, increasing the number of siliques on each plant will lead to an increase in seed production per plant [14,15]. 1000-SW and FSW were also shown to have a statistically significant and favorable relationship with seed production. So, these became the key players in determining seed production [16,17]. When plants are under water stress, their leaf growth rate drops [18], their stomata shut, and their photosynthetic rate drops, all of which contribute to smaller seeds and a lower weight per 1000 seeds [19,20].

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